Claims

1. A screening method for simultaneous detection of diarrheagenic *Shigella spp.* and *E. coli* (DEC) including A/EEC & EPEC, ETEC, VTEC, EIEC and strains with the *ehxA* gene.

2. A screening method according to claim 1, which detects the presence of the genes *ehxA, eae, vtx1, vtx2, ipaH, sta, elt* and *bfpA.*

3. A screening method according to claim 1-2 for detecting *Shigella spp.* by detecting the presence of the *ipaH* gene.

4. A screening method according to claim 1-3 performed with multiplex PCR.

5. A screening method according to claim 4, using the primers selected from table 3.

6. A screening method according to claim 4-5, which additionally incorporates a positive control.

7. A screening method according to claim 6, where the positive control is 16S rDNA.

8. A screening method according to claim 4-7, which uses the UNG system.

9. A screening method according to any of the preceeding claims where the genes are detected by size identification, e.g. by agarose gel electrophoresis or capillary electrophoresis.

10. A screening method according to any of the preceeding claims where the genes are detected with a hybridisation probe.

11. A screening method according to any of the preceeding claims where the material to be analysed can be any material from where bacteria can be extracted, e.g stool samples, consumables etc.

12. An *in vitro* diagnostic method for determining the risk of being infected with a pathogenic organism giving rise to haemolytic uremic syndrome (HUS) or hemorrhagic colitis, said method comprising detecting the *ehxA* gene in the DEC.

13. A method for simultaneously detection of diarrheagenic *E. coli* (DEC) groups A/EEC & EPEC, ETEC, VTEC, and EIEC by testing for the presence of the genes: *ipaH, eae, ehxA* and *sta*, parts of these genes or products of these genes or parts thereof, such as RNA or polypeptides.

14. A method according to claim 13, which further comprises (simultaneously) testing for the presence of one or more (such as more than 2, 3 or 4) of the genes selected from the group comprising: *vtx1, vtx2, elt*, and *bfpA*, parts of these genes or products of these genes or parts thereof, such as RNA or polypeptides.

15. A method according to claim 14, detecting the genes selected from the group comprising: *ipaH, eae, ehxA, sta, vtx1, vtx2, elt,* and *bfpA,* parts of these genes or products of these genes or parts thereof, such as RNA or polypeptides.

16. A method according to claim 14, detecting the genes selected from the group comprising: *ipaH, eae, sta, vtx1, vtx2,* and *elt,* parts of these genes or products of these genes or parts thereof, such as RNA or polypeptides.

17. A method of any of claims 13-16, in which the testing is carried out on a sample, such as a sample from a human or an animal (ie. stool), a sample from a consumable products (ie. food and beverages), a bacteria culture or a sample from sewage.

18. A method of any of claims 13-17, in which the testing is carried out using a nucleotide sequence amplification technique, such as PCR, multiplex PCR, real-time PCR.

19. A method according to any of the preceding claims, in which at least one primer used is selected from the group consisting of:
a) the primers of table 3,
b) sequences having a sequence identity of at least 80% (such as a least 85%, at least 90%, or at least 95%) with the primer sequences of a)
c) parts of the sequences in a) or b), having a lenght of more than 10, preferred more than 13 nucleotides (eg. consisting of 14, 15, 16, 17, 18, 19, 20, 21 or 22 consequtive nucleotides),
d) sequences comprising a sequence in a), b) or c), said sequence having a lenght of at least most 100 nucleotides, such as at most 90, 80, 70, 60, 50, 40, or at most 30 nucleotides.

20. A method according to any of the preceding claims, which additionally incorporates a positive control.

21. A method according to any of the preceding claims, where the positive control is 16S rDNA.

22. A method according to any of the preceding claims, which uses the UNG system.

23. A method according to any of the preceding claims, wherein the genes are detected by size identification, e.g. by agarose gel electrophoresis or capillary electrophoresis.

24. A method according to any of the preceding claims, wherein at least one product of the nucleotide sequence amplification reaction is detected with a hybridisation probe.

25. A nucleotide sequence selected from the group consisting of:
a) the primer sequences of table 3,
b) sequences having a sequence identity of at least 80% (such as a least 85%, at least 90%, or at least 95%) with the primer sequences of a)
c) parts of the sequences in a) or b), having a lenght of more than 10, preferred more than 16 nucleotides, such as more than 17, 18, 19 or 20 nucleotides (eg. consisting of

14, 15, 16, 17, 18, 19, 20, 21 or 22 consequtive nucleotides of the sequences in a) or b)),
d) sequences comprising a sequence in a), b) or c), said sequence having a lenght of at least most 100 nucleotides, such as at most 90, 80, 70, 60, 50, 40, or at most 30 nucleotides.

26. A nucleotide sequence, which is selected from the primer sequences of table 3.

27. A nucleotide sequence selected from the group consisting of:
a) the probe sequences of table 7,
b) sequences having a sequence identity of at least 80% (such as a least 85%, at least 90%, or at least 95%) with the primer sequences of a)
c) parts of the sequences in a) or b), having a lenght of more than 10, preferred more than 16 nucleotides, such as more than 17, 18, 19 or 20 nucleotides (eg. consisting of 14, 15, 16, 17, 18, 19, 20, 21 or 22 consequtive nucleotides of the sequences in a) or b)),
d) sequences comprising a sequence in a), b) or c), said sequence having a lenght of at least most 100 nucleotides, such as at most 90, 80, 70, 60, 50, 40, or at most 30 nucleotides.

28. A nucleotide sequence which is selected from the probe sequences of table 7.

29. A kit which comprises, in a single or in separate containers, nucleotide sequences which are able to prime amplification in a nucleotide sequence amplification reaction, such as PCR, of the genes: *ipaH, eae, ehxA*, and *st,* or parts of these genes or the complementary strands to the genes or parts thereof.

30. A kit which comprises, in a single or in separate containers, nucleotide sequences which are able to hybridise (preferably under stringent conditions) with the genes: *ipaH, eae, ehxA*, and *sta*, parts of these genes or the complementary strands to the genes or parts thereof.

31. A kit according to any of the preceding claims, which comprises at least one (such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) nucleotide sequence of claim 25-26.

32. A kit according to any of the preceding claims, which comprises a means for detection, such as a probe with a nucleotide sequence according to claim 27-28.

33. A kit according to any of the preceding claims, which comprises a means for amplification of a nucleotide sequence, such as a polymerase or nucleotides.

34. A kit according to any of the preceding claims, which comprises a means for a control, such as primers for 16S rDNA.

35. A kit according to any of the preceding claims, which comprises a means for detecting by size identification, ie. an agarose gel or a capillary tube optionally filled with buffer.

36. A kit according to any of the preceding claims, which comprises nucleotide sequences which are able to prime amplification of at least one gene selected from the group consisting of : *vtx1, vtx2, elt,* and *bfpA*, or parts of these genes or the complementary strands to the genes or parts thereof.

37. A kit according to claim 36, in which the nucleotide sequences for priming are selected from the group consisting of the priming sequences in table 3.

38. A kit according to claim 36, in which the nucleotide sequences for probing are selected from the group consisting of the probe sequences in table 7.